Activation regulated gene circuit for controlling payload expression in cell therapies

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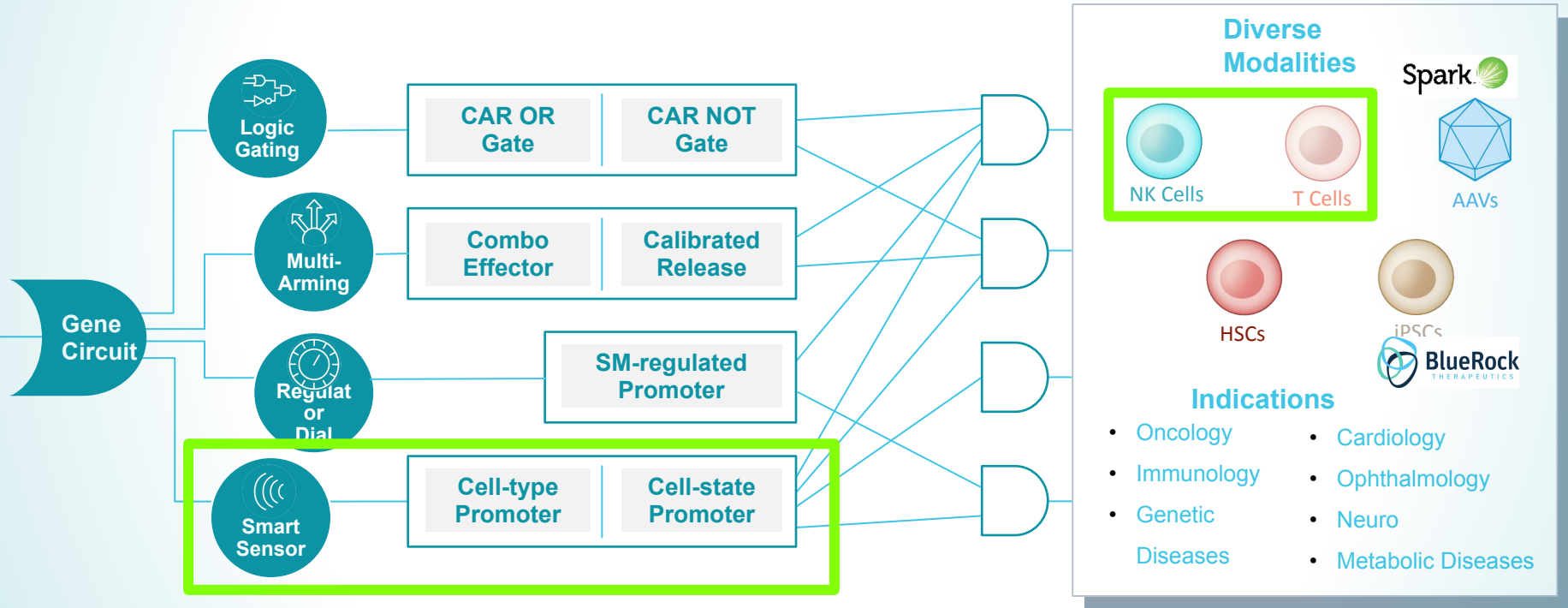
Presenter: Michelle Hung

05.16.2022

1

Disclosures

Michelle Hung is a paid employee of Senti Biosciences

Gene Circuits Could Potentially Power Multiple Cell and Gene Therapy Modalities for Broad Therapeutic Potential
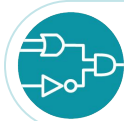
Senti's Gene Circuits can be designed to overcome key hurdles in cell based immunotherapy for solid tumors

**REPRESENTATIVE
CLINICAL MANIFESTATION**

…ARE TACKLED THROUGH INTELLIGENT GENETIC PROGRAMMING

Clinical Manifestation	Programming	Description
On-Target Off-Tumor Tox	LOGIC GATING	Integrates multiple targets to pinpoint diseased cells and spare healthy ones
Tumor Relapse	MULTI-ARMING	Targets multiple disease pathways within a single all-in-one drug
CRS / ICANS / Systemic Immune Toxicity	REGULATOR DIAL	Dynamically regulates therapies *in vivo* using FDA-approved oral drugs
Off-Tumor Tox/Systemic Immune Toxicity	SMART SENSOR	Precisely detects and responds to disease environments

Existing sensors have had limited success due to high basal activity
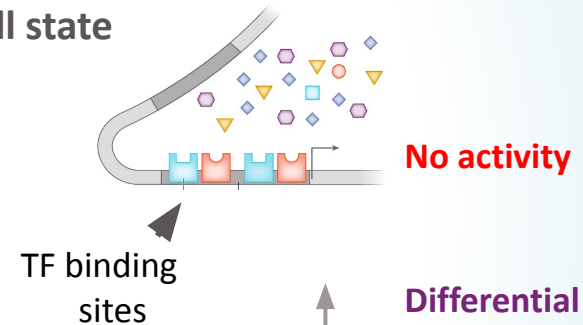
First Generation NFAT Promoter Sensor

o NFAT-regulated promoters have long been used as reporters for T and CAR-T activation

o A 2015 trial studied NFAT-regulated IL-12 in TILs, but the **trial was suspended** because of **clinical toxicities** due to **high basal expression of IL-12 in absence of activation cue**
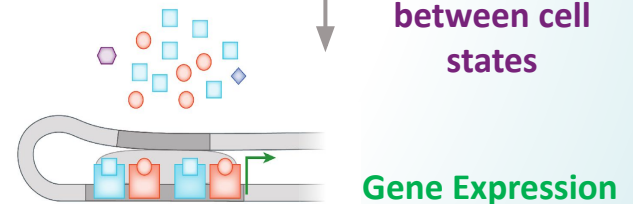
Ideal Next Generation Smart Sensors

o Smart Sensors for T and NK cells with
  - high expression in activated cells
  - low to no basal expression in resting cells

Senti's Approach to Generate Smart Sensors
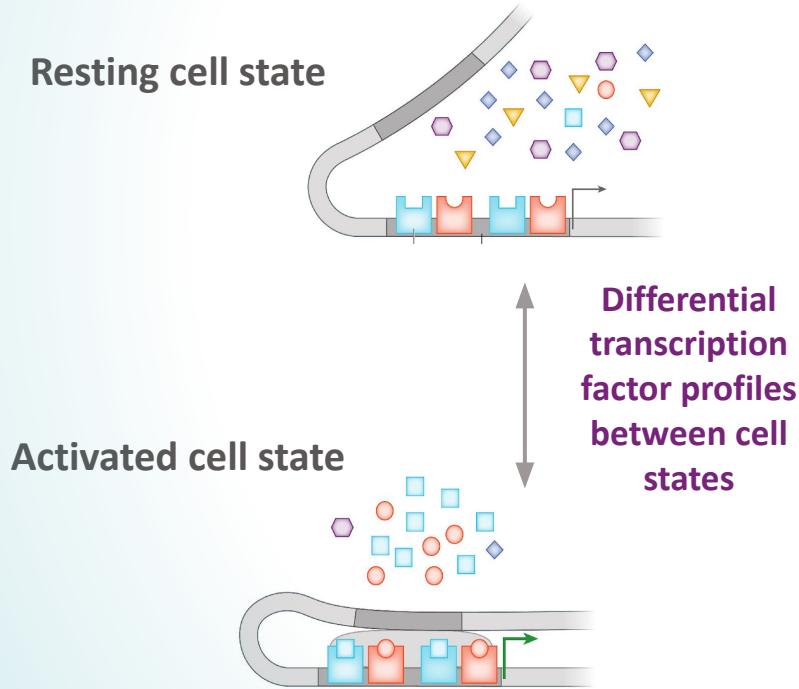
Resting cell state

No activity

TF binding sites

Differential transcription factor profiles between cell states

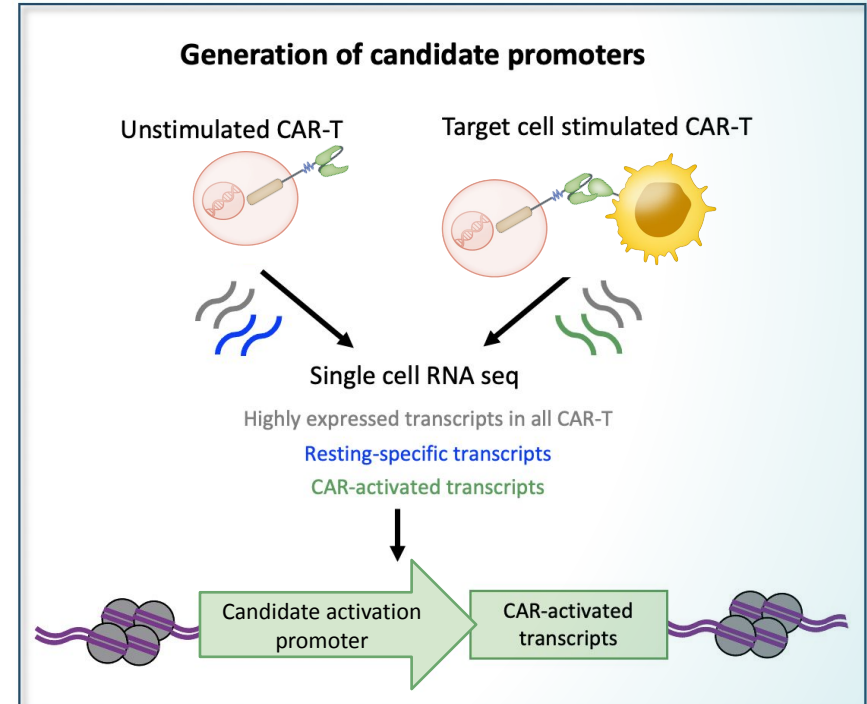**Activated cell state**

Gene Expression

RNA Seq can identify transcriptional differences between resting and activated cells

Smart Sensors Rely on Different Transcriptional States

Resting cell state

Activated cell state

Differential transcription factor profiles between cell states

Senti's Screen for T Cell Activation Transcriptional Changes

Generation of candidate promoters

Unstimulated CAR-T

Target cell stimulated CAR-T

Single cell RNA seq

Highly expressed transcripts in all CAR-T

Resting-specific transcripts

CAR-activated transcripts

Candidate activation promoter

CAR-activated transcripts

Senti is developing Smart Sensors so that engineered immune cells deliver potent immuno-regulators with minimal basal expression

Experimental Design

Results

Candidate promoter → Fluorescent Reporter

Lentivirally transduce into primary T cells

Rest 14 days, then +/- activation

No activation

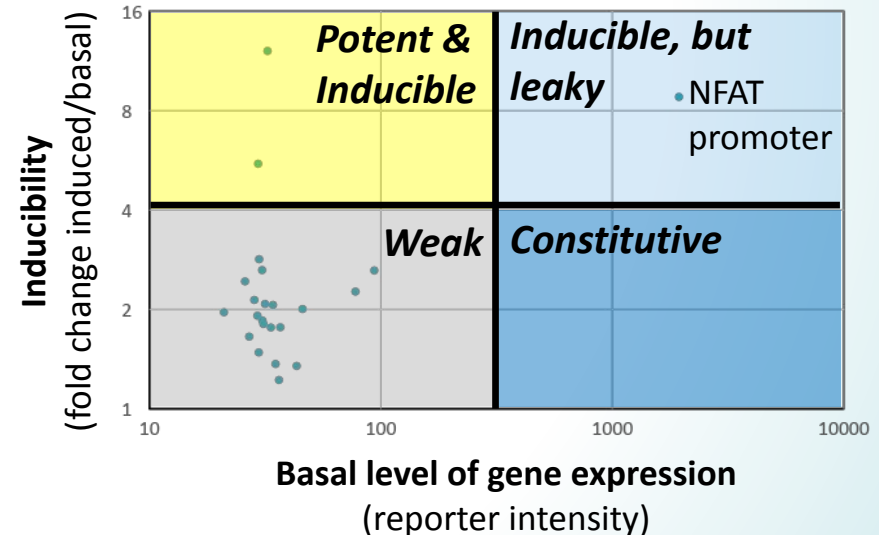Activation (CD3/CD28 dynabeads)

Basal level of gene expression

Induced level of gene expression

Potent & Inducible

Inducible, but leaky · NFAT promoter

Weak

Constitutive

Inducibility (fold change induced/basal)

Basal level of gene expression (reporter intensity)

Senti is developing Smart Sensors so that engineered immune cells deliver potent immuno-regulators with minimal basal expression

Experimental Design

Candidate promoter → Fluorescent Reporter

Lentivirally transduce into primary T cells
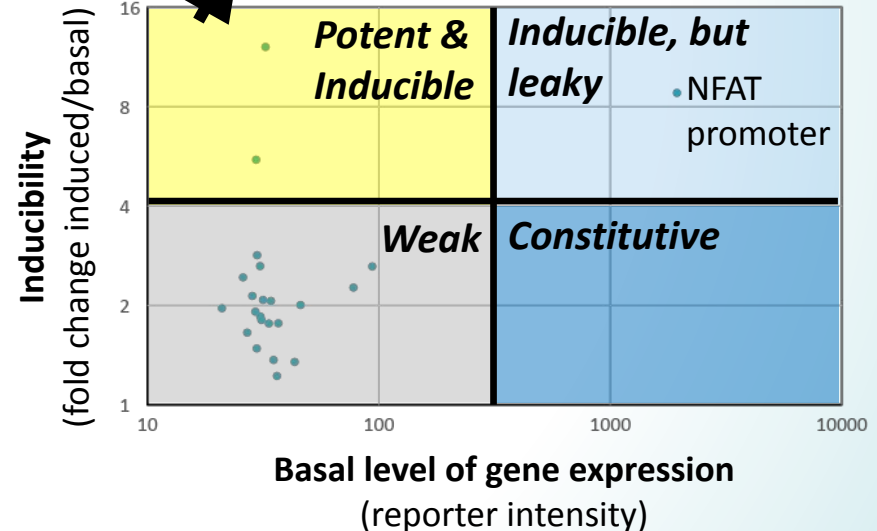

Rest 14 days, then +/- activation

No activation

Activation (CD3/CD28 dynabeads)

Basal level of gene expression

Induced level of gene expression

Results

Promoter A – feed into Design, Build, Test, Learn cycle to improve performance

Inducibility (fold change induced/basal)

Potent & Inducible

Inducible, but leaky • NFAT promoter

Weak

Constitutive

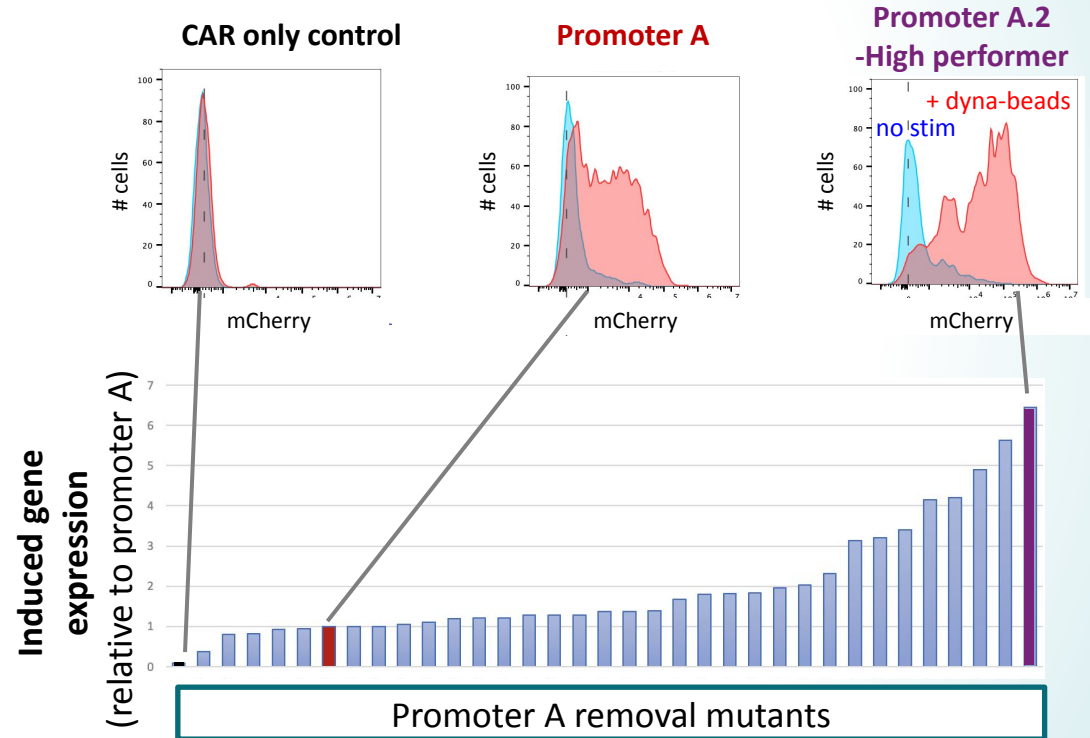**Basal level of gene expression** (reporter intensity)

8

Within the lead promoter, regulatory elements were bioinformatically identified and removed to identify their functions
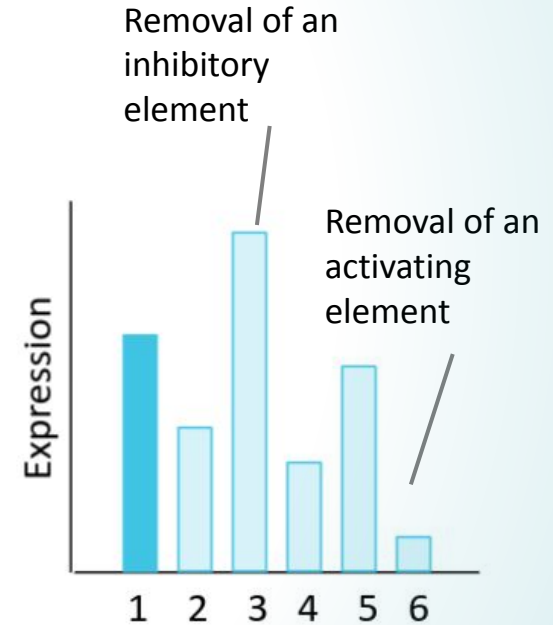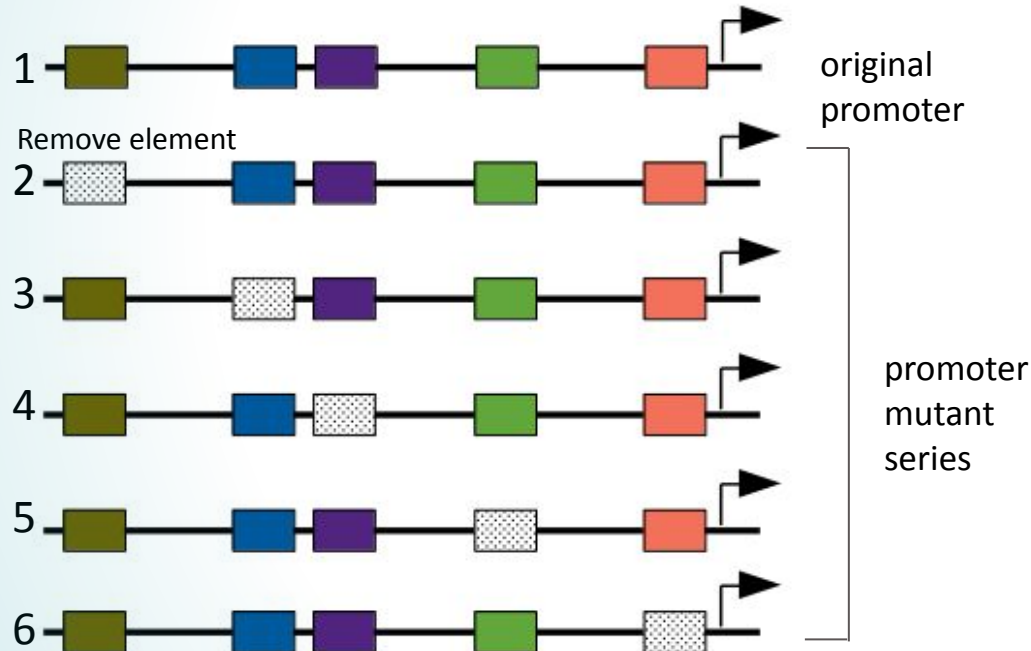
Systematic removal of regulatory elements yielded several promoters with improved performance

- By screening dozens of mutants, **a set of promoters were discovered that exhibited superior inducibility**

- Systematic removal yielded a functional map of the promoter, identifying regions associated with inducible and leaky expression

CAR only control

Promoter A

Promoter A.2 -High performer

+ dyna-beads

no stim

mCherry

mCherry

mCherry

cells

Induced gene expression (relative to promoter A)

Promoter A removal mutants

Rational combination of regulatory elements yields smaller, more potent promoters
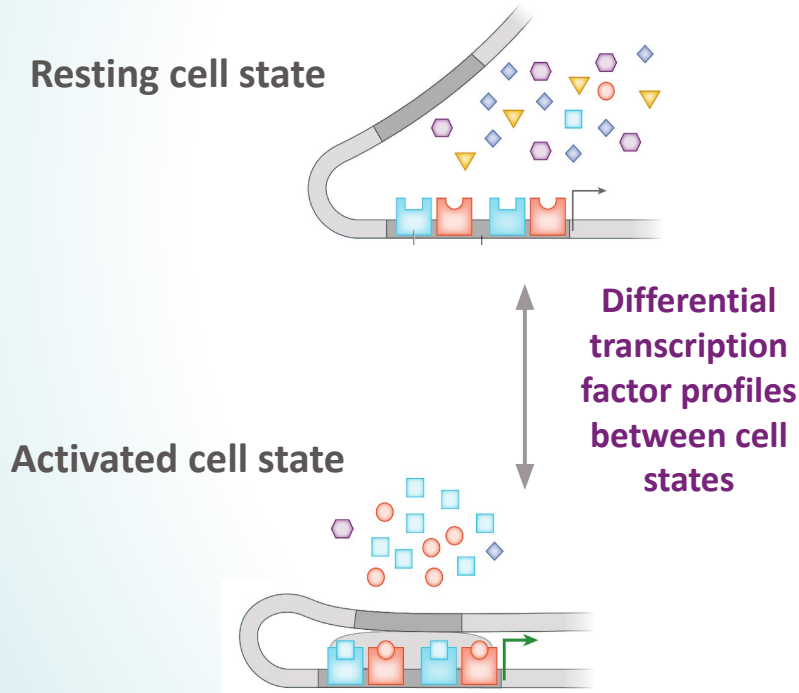
- New promoters were generated by deleting inhibitory regulatory elements, alone or in combination

- This led to the generation of smaller promoters, several with similar or better activation induction than the top removal promoter

★ Promoter A.3

(40% smaller & 250% activity relative to promoter A.2!)
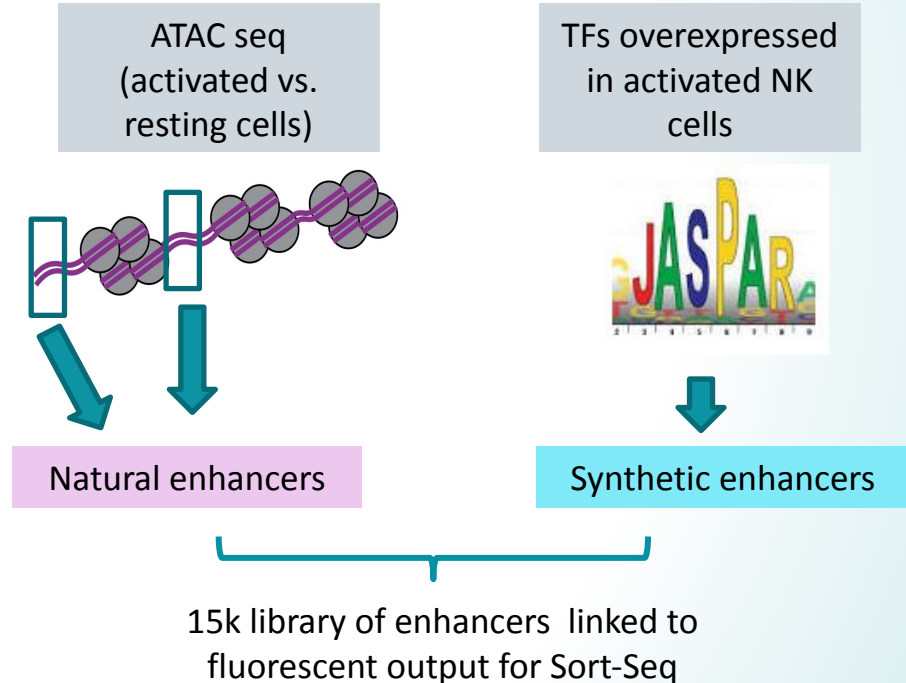
■ Promoter A.2

(best mutant of promoter A)
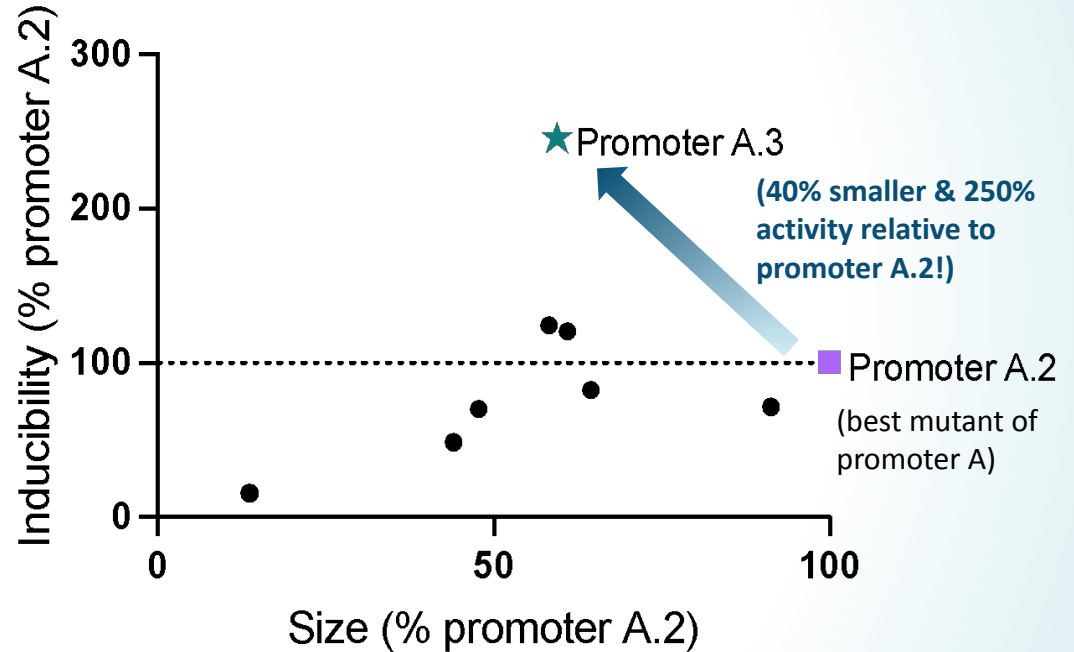
...nti has discovered Smart Sensors with low basal expression and high inducibility and further optimized one such sensor using our Design, Build, Test, Learn Cycle

Senti has generated a promoter library that targets transcriptional differences between resting and active NK cells

Smart Sensors Rely on Different Transcriptional States

Resting cell state

Differential transcription factor profiles between cell states

Activated cell state

Senti's Screen for NK Activation Induced Promoters

ATAC seq (activated vs. resting cells)

TFs overexpressed in activated NK cells

Natural enhancers

Synthetic enhancers

15k library of enhancers linked to fluorescent output for Sort-Seq
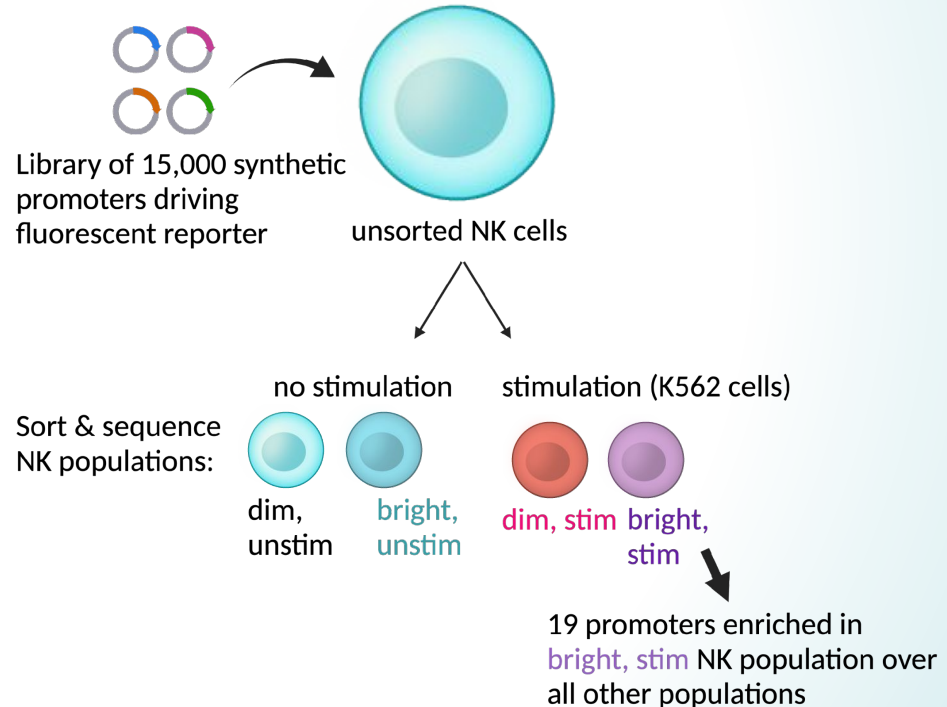
Senti has screened a promoter library that targets transcriptional differences between resting and active NK cells

Promoter Library Design

variable region (200 bp)

Minimal promoter
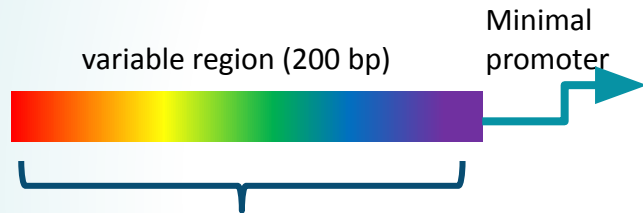
- 200bp regions of activation associated ATAC seq open chromatin regions (putative enhancers)

- Arrays of activation associated transcription factor binding sites (2TFs x 4 sites)
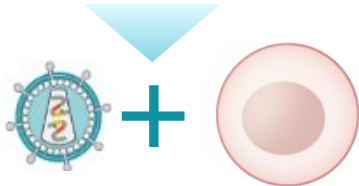
High Throughput Promoter Screen

Library of 15,000 synthetic promoters driving fluorescent reporter

unsorted NK cells

no stimulation

stimulation (K562 cells)

Sort & sequence NK populations:

dim, unstim

bright, unstim

dim, stim   bright, stim

19 promoters enriched in bright, stim NK population over all other populations

13

Senti has identified 3 top promoters from the library via clonal validation

Experimental Design

Candidate promoters from HTP screen → Fluorescent Reporter

Retrovirally transduce into primary NK cells
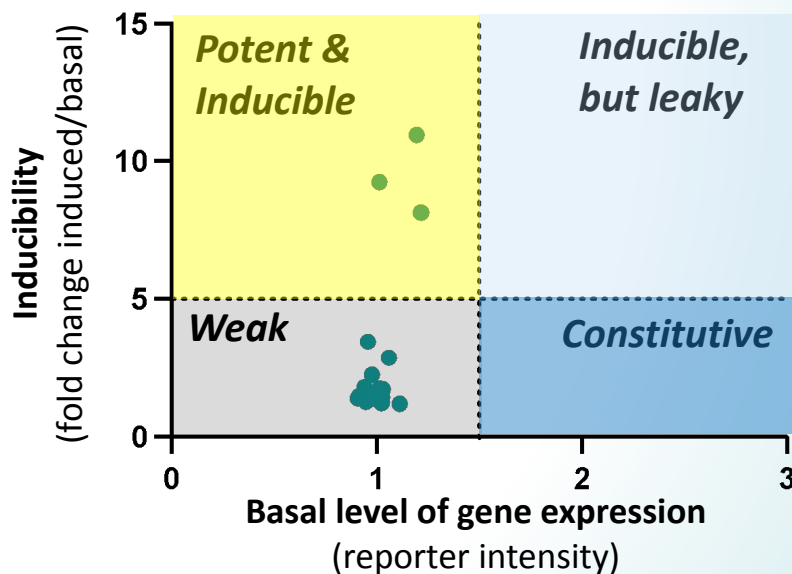
Rest 7 days, then +/- activation

No activation

Activation (K562 cells)

Basal level of gene expression

Induced level of gene expression

Results

- Y-axis: **Inducibility** (fold change induced/basal), 0 to 15
- X-axis: **Basal level of gene expression** (reporter intensity), 0 to 3

Quadrant labels:
- *Potent & Inducible*
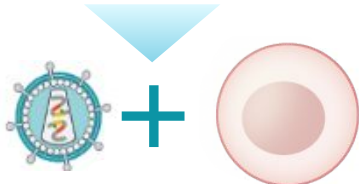- *Inducible, but leaky*
- *Weak*
- *Constitutive*

Senti has identified 3 top promoters from the library via clonal validation

Experimental Design

Candidate promoters from HTP screen

Fluorescent Reporter

Retrovirally transduce into primary NK cells

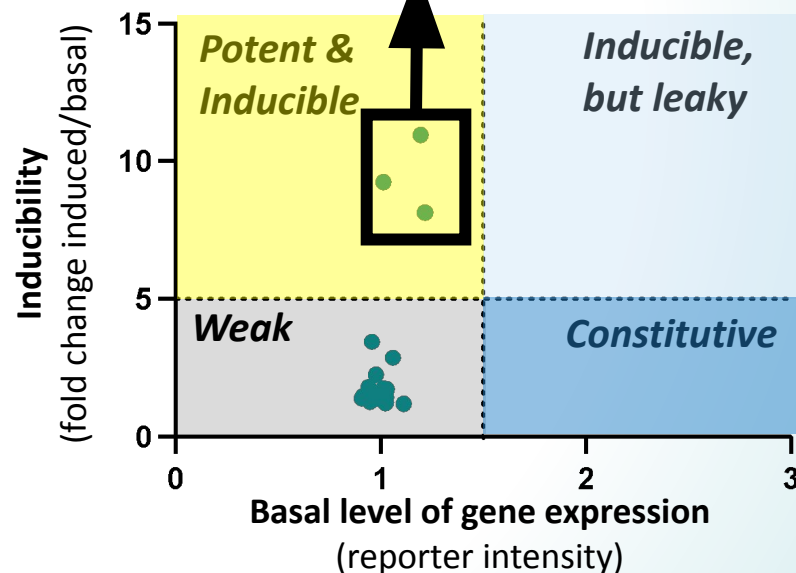Rest 7 days, then +/- activation

No activation

Activation (K562 cells)

Basal level of gene expression

Induced level of gene expression

Results

Compact (200bp) promoter leads to undergo future Design, Build, Test Learn Cycles

Potent & Inducible

Inducible, but leaky

Weak

Constitutive

Inducibility (fold change induced/basal)

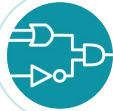**Basal level of gene expression** (reporter intensity)

15

Senti's Smart Sensors enable immune cells to produce payloads only when in the activating tumor environment

REPRESENTATIVE CLINICAL MANIFESTATION		...ARE TACKLED THROUGH INTELLIGENT GENETIC PROGRAMMING	
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Off-Tumor Tox/Systemic Immune Toxicity	▶	SMART SENSOR	Precisely detects and responds to disease environments

16

Acknowledgements

Thank you to the fantastic team at Senti Biosciences!

See our other Senti Presentations:
- Title: Logic Gated FLT3 OR CD33 NOT EMCN CAR-NK Cell Therapy (SENTI-202) for Precise Targeting of AML
  Garrison et al. (abstract 844) – May 18 at 3:45-5:30 PM
- Title: Multi-Arming and Regulator Dial Gene Circuits to Address Key Disease Challenges in HCC
  Guzman Ayala et al. (abstract 352) – May 16 at 5:30-6:30 PM